PME RESEARCH LABORATORY, JUNE 1980

PROJECT TITLE : PROTAGORAS

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WRITTEN BY : A. HAENGGI

The aim of the project is the determination of the influences proteins of tobacco have on the smoke composition, on smoke condensate and on cigarette subjectives. Our present problems are the extraction of proteins from tobacco and the preparation of reconstituted tobacco sheets.

1. PROTEIN EXTRACTION FROM TOBACCO DUST USING ENZYMES (1)

Comparison of the proteolytic power of 9 commercially available enzymes.

Procedure : 180 g tobacco dust (13.1 % proteins)
+ 500 ml water
↓
pH adjusted with HCl or NaOH
+ 200 mg enzymes
↓
incubation at 170 r.p.m. during 6 hours
↓
centrifugation at 20'000 r.p.m. during 30 minutes
↓
supernatent analysis

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Enzyme	supplier	source	temp.	pH	% protein extraction
Protease	Boehringer	bacterium	37°C	7.5	38.65
Protease	Calbiochem	"	37°C	7.5	55.99
Trypsin	Fluka	--	37°C	7.5	34.97
Proteolytic	ICN	--	37°C	7.5	38.36
Proteinase	ICN	--	37°C	7.5	37.70
Neutrase	Novo	bacterium	55°C	7.5	41.19
Alcalase	Novo	"	55°C	7.5	50.17
Pepsin	Fluka	--	37°C	1.8	47.25
Papain	Calbiochem	plant	37°C	7.5	38.10

2. PROTEIN EXTRACTION FROM BURLEY STRIPS (2)

Burley strips containing 20 % proteins have been extracted under the same conditions using Calbiochem protease and Trypsin as proteolytic enzymes.

Enzyme	protein extraction in %
Protease (Calbiochem)	59.4
Trypsin (Fluka)	41.1

3. OPTIMALIZATION - PROTEASE (3)

The results obtained from the comparison of the enzymes show that the proteolytic power of the Calbiochem Protease is most efficient for our purposes. In order to improve its catalytic activity different parameters have to be optimized.

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In a first step the influence of the incubation temperature was tested. All other parameters mentioned under point I remained unchanged.

Temperature	% protein extraction
30°C	51.4
37°C	54.45
44°C	56.20
51°C	-- (58.29*)
58°C	66.14 (61.42*)
65°C	69.20 (65.23*)
72°C	66.26

* only 150 mg enzymes instead of 200 mg.

A maximum yield of the extraction is obtained at a temperature of 65°C. At higher temperatures, a decrease of the extraction efficiency can be observed which is certainly caused by the destruction of the enzyme.

4. FUTURE WORK

- Continuation of Protease optimalization (incubation time, enzyme concentration, pH)
- Utilization of a cigarette tobacco mixture (B-blend - type MLF) (4)

REFERENCES

(1) A. Hänggi, Notebook 791203, pp. 9-14 + 17
(2) M.F. Mangilli, Notebook 791205, pp. 38-39
(3) A. Hänggi, Notebook 791203, pp. 15-16 + 20-25
(4) F. Moser, Project Report Spotless, January 1980.

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